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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Bror Morein and Karin Lovgren Bengtsson

Serial No.: 10/520,022

Date Filed: January 23, 2006

For: ISCOM PREPARATION AND USE THEREOF

I hereby certify that this paper is being facsimile transmitted (571) 273-8300 or deposited this date with the U.S. Postal Service as first class mail addressed to:

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Robert D. Katz 6/6/08
Robert D. Katz Date
Reg. No. 30,141

1185 Avenue of the Americas
New York, N.Y. 10036
June 6, 2008

Sir:

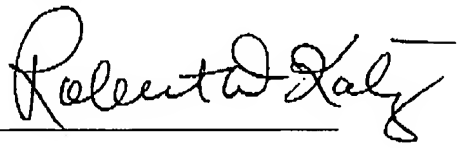
COMMUNICATION FILING DECLARATION UNDER 37 C.F.R. §1.132

Applicants file herewith a Declaration Under 37 C.F.R. §1.132 of Karin Lovgren Bengtsson, Ph.D., one of the co-inventors herein. The declaration addresses the Cox patent cited by the Examiner, and makes plain that the claimed invention is non-obvious and therefore patentable over the cited reference. Applicants have learned that the adjuvant discussed in the reference displays undesirable toxicity, while the adjuvant of the claimed invention displays superior and unexpected adjuvant properties, with markedly reduced toxicity. For these principal reasons, Applicants submit that the claimed invention is patentable over the references of record, and respectfully request that the outstanding obviousness rejection be reconsidered and withdrawn.

The Director is authorized to charge any fee required in connection with this response to Deposit Account No. 03-3125. If any extension is required in connection with the filing of this response, applicants hereby request same and authorize the fee therefor to be charged to Deposit Account No. 03-3125.

Dated: June 6, 2008

Respectfully submitted,



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Bror Morein
Serial No.:	10/520,022
Date Filed:	January 23, 2006
For:	ISCOM PREPARATION AND USE THEREOF

DECLARATION OF KARIN LOVGREN BENGTSSON, Ph.D.,
PURSUANT TO 37 C.F.R. §1.132

Karin Lovgren Bengtsson, Ph.D. declares as follows:

1. I one of the named inventors of the above-identified application.I have a B. Sci. in Biochemistry, Uppsala University, Uppsala, Sweden; a Ph.D. in Vaccinology from the University of Agricultural Sciences, Uppsala, Sweden. I am currently Associate Professor at the University of Agricultural Sciences, Uppsala Sweden. My thesis involved research on ISCOM technology. I am the co-inventor of nine patent families and the co-author of about 80 publications, all relating to the ISCOM technology.

2. I understand that the Examiner has rejected the claims of our patent application, asserting that it would have been obvious to a person of ordinary skill in the art based upon the cited Cox patent. I respectfully disagree with the Examiner's rejection, and will explain in this Declaration why I believe that the claimed invention is nonobvious.

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3. By way of background, during the latter half of the 1980's a lot of effort was spent by our group and others on the separation and purification of Quillaja saponins for use as adjuvants. Many of the side-reactions seen with the use of saponins were attributed or blamed on the heterogeneity and the lack of knowledge regarding the function of individual saponin molecules, especially the hemolytic structures.

4. ISCOM contains at least one glycoside, at least one lipid and at least one type of antigen substance. An ISCOM matrix comprises at least one glycoside and at least one lipid. The glycoside may be a saponin from Quillaja Saponaria Molina.

5. The fractions of saponin represent material with similar properties regarding adjuvant activity and structure-forming (ISCOM/Matrix) capacity. For example, fraction-A saponins are virtually non-toxic and can build ISCOM/Matrix structures. Their adjuvant activity were regarded as very low (based on their inability to enhance and promote antibody production), compared with the Fraction-B and Fraction-C saponins. Fraction-B saponins were found to be very hemolytic and toxic, so it was decided to work with the Fraction-C saponins as major source of adjuvant active purified saponins. Even though the toxicity of the Fraction-C saponins was lower than those of the omitted Fraction-B saponins, toxicity was still an issue, particularly in sensitive animals such as mice. This was especially so when working with the classical ISCOM-technology, in which the antigens were physically integrated into the particles formed by saponin, cholesterol and

phospholipids. In order to integrate a substantial amount of antigen into the ISCOM particles, Fraction-C saponin is needed for the construct itself. This made it technically difficult to steer the ratio of antigen to adjuvant (Fraction-C) in the construct and hence the amount of saponin molecules often became higher than necessary and higher than tolerated for many antigen formulations.

6. The cited Cox patent was aimed as a solution to these problems. Considering that the Fraction-A saponins were non-toxic and well suited for particle formation, the idea was to "dilute" the adjuvant active Fraction-C saponins with non-toxic building blocks of Fraction-A in order to control the antigen:Fraction-C composition. We found that up to 70% of the Fraction-C blend could be exchanged with the non-adjuvant active Fraction-A saponins without losing adjuvant activity. Since the basic idea was ISCOM particles together with the Fraction-C saponins, we never considered or tried to form separate particles made from the different saponin fractions. That would not simply serve the purpose of creating less toxic (reactive) ISCOM particles with a controlled amount of antigen and Fraction-C. The ideal blend of Fraction-A and Fraction-C was defined as a mixture consisting of 70% Fraction-A and 30% Fraction-C. The saponin mixture was given the name ISCOPREP 703. At the time of filing the present application, no safety studies had yet been performed on that product.

7. Years later, our group in Sweden was still working with different saponin fractions.

We found that the Fraction-A saponin was not as adjuvant inactive as we first thought. Fraction-A saponin was still not good in stimulating antibody responses, but was interestingly potent in stimulation or activation of cellular responses (Johansson and Lövgren-Bengtsson, "ISCOMs with Different Quillaja Saponin Components Differ in Their Immunomodulating Activities," Vaccine, 1999, 17, 2894). With such diverse activities, we got the idea of forming ISCOM/Matrix particles with the pure saponin fractions respectively and then after formation mixing the separate particles in order to see if we could get new adjuvant properties. The mixture of separate ISCOM/Matrix particles made from Fraction-A and Fraction-C saponins respectively seemed to exhibit many similar activities as the prototype 703 and Fraction-C formulations. There were two important exceptions: (1) the "full adjuvant activity" seemed to be achieved with a lower amount of Fraction-C material added (as separate particles); and (2) most surprisingly, the formulation exhibited a much lower toxicity. In fact, the side effects were reduced to a level under what was expected (but never obtained) with the ISCOPREP 703 formulation. It was later shown in vitro that the admix of Fraction-A particles to the Fraction-C particles even has a protective effect lowering the toxicity of the Fraction-C particles (see Table 1, attached).

The Results Presented in the Cox Patent (Patent No. U.S. 6,352,697)

8. Example 1 of USP 6,352,697 relates only to the purification of Fractions A, B and C of Quil A. In Example 2, ISCOM matrixes are prepared from Fraction A and Fraction C alone or in combination (mixed together before ISCOM/matrix formation).

9. This example was performed to evaluate the optimal concentration of the ingredients to produce whole ISCOM particles and not only fragments thereof. However, it nowhere states that Fraction A alone in one ISCOM matrix particle should be combined with Fraction C alone in another ISCOM matrix particle.

10. In Example 3, ISCOM's are prepared containing influenza antigen. The mice groups were immunized with ISCOMs comprising Fraction A only (groups 1-4); ISCOMs comprising Fraction C only (groups 5-8); ISCOMs comprising Fraction C only (groups 9-12) and ISCOMs comprising both Fraction A and Fraction C in a ratio 7: 3 (groups 13-16) (see Table 4 and column 9, lines 42-44). The two-saponin fractions are mixed together prior to particle formation. Even though ISCOM comprises separate fractions of Quil A were prepared, they were only used for comparison with ISCOM's comprising both Fraction A and Fraction C. Consequently, ISCOM's comprising separate fractions of Quil A were never tested and immunized as mixtures together. Such a formulation is also not designed, mentioned or suggested in the Cox patent.

11. In Examples 4, 5, 7 and 8 the 7:3 mixture of Fraction A and C is tested in combination with different antigens for the preparation of ISCOMs. In example 6, the induction of IL-1 of ISCOM matrices comprising Fraction A only, Fraction C only and mixtures thereof together in ISCOM matrices are tested. In Example 7, the hemolytic activity of various saponins in solution and in ISCOM's is tested. In Example 7 (column 12, lines 32-39):

> However, when these saponins are used to make ISCOMs, the degree to which the hemolytic activity is decreased is variable ranging from about 10 fold for QH-B(Fraction -B) and QH-C (Fraction -C) and 40 fold for QH-A (Fraction A) and QH703. It is therefore demonstrated that the use of QH703 in ISCOMs is an optimal way to incorporate adjuvant active quantities of QH-C (Fraction C) whilst minimizing the hemolytic activity of this saponin.

12. Therefore, the mixture of Fraction A and fraction C in a ratio of 7:3 permits at least the same immunogenicity and efficacy as shown by the more toxic Fractions of Quil A, but with a much lower level of those toxic components (column 10, lines 5-8).

13. Nowhere in this document are any immunization tests performed with a mixture of different ISCOM or ISCOM matrix particles comprising different Fractions of Quil A. This is also not mentioned or suggested anywhere in the Cox Patent.

Lethal Dose According to the Cox patent

14. No data regarding toxicity is presented in the Cox patent. On the contrary, the comparatively high IL-1 production stimulated by 703 Matrix (Example 6, fig. 5 and especially column 11 lines 62-26 of the Cox Patent) can be compatible with the surprisingly high toxicity that has been recorded with 703 preparations. The high toxicity of 703 formulations and the failure to reduce the toxicity and side effects by the 703 formulations are indeed unexpected. In column 13, lines 28-36 it is states:

> The conclusions from the single dose toxicological studies were that it was difficult to clearly establish the "No Toxic Effect Level" in these studies, with QH703 and ISCOM matrix prepared from QH703 showing limited non-specific lethality in high doses (10 mg.kg. -1 and 1.4 mg.kg. -1 respectively). These doses represent greater than 1000 times the dose, which is believed to be the maximum therapeutic dose.

This statement must be related to an estimated human dose since the dose levels usually (and also in the Cox-patent) used in mice, i.e., 5-10 micrograms per ≈ 20 gram mouse correspond to about 0,25 – 0,5 mg/kg, which is only 2 – 5-fold less that the lethal dose of 1,4 mg/kg.

The present invention US 10/520022

15. The present invention relates primarily to a method of decreasing the toxicity (the side effects) of mixtures of Quillaja saponin fractions (sub fragments of Quil A).

This is accomplished by making different ISCOM/ISCOM-matrix particles containing only one fraction (sub fragment of Quil A) each and then mixing the individual ISCOM/ISCOM-matrix particles. Such a formulation is fundamentally different than the ones described in the Cox Patent.

Mortality and Toxicity

16. The present patent teaches that when two saponin fractions, i.e. Fraction-A and Fraction-C are formulated in physically separate particles, the toxicity of this formulation is substantially reduced and can be administered to the vaccine with high degree of comfort. This was indeed unexpected. Concretely, Balb/c mice tolerate doses of the formulations according to the present invention (group 1 in Table 2 on page 17 of the published application WO 2004/004762) that are lethal if these fractions are combined in the same particles (group 8 in Table 2).

17. Thus, in Example 4 Balb/C mice were immunized days 0 and 42 with a mixture of different ISCOM matrix particles comprising Fraction A and Fraction C of Quil A in separate particles (MIX groups 1, 2 and 3) and compared with, the ISCOM matrix with both Fraction A and Fraction C in the same particle (CONV groups 7, 8, 9, 10 and 11 i.e. as in the Cox patent), or with ISCOM matrix containing 100% of Fraction A (groups 4 and 5) or 100% of Fraction C (group 6 and 12). In Table 2 the expression CONV relates to Fraction A and Fraction C in the same ISCOM

matrix particle. Clearly, only the low dose of 10µg of Fraction A and Fraction C (only 3µg Fraction C, 30% of 10 µg) of Quil A in a mixture in an ISCOM matrix particle (CONV group 8) has an acceptable surveillance survival rate (0 out of 8 mice). The rest of the groups receiving 50µg of different combinations of Fraction A and C of Quil A in an ISCOM matrix particle (CONV groups 9-12) had too many dead 5-8 out of all 8 mice. Most of the mice in group 9-12 were dead and were therefore not included in the rest of the immunological tests.

18. In contrast, when a total of 50 µg of Fraction A and C were incorporated in different ISCOM matrix particles only 0 to 2 mice out of 8 died (groups 1-3). Thus, the mice were more sensitive to Fraction C when combined with Fraction A in the same CONV matrix particle (groups, 8 9, and 10) than when combined in different particles according to the present invention (groups 1-3).

19. Enclosure 1 shows lethargy (describes side-effects and "not feeling well") and deaths tests performed with ISCOM particles comprising OVA antigen (egg albumin) and Fraction A in one ISCOM particle and OVA and Fraction C of Quil A in another particle (ISCOM–A+ISCOM-C 83:17) in Table 2 of Enclosure 1. This is compared with ISCOM -AC (83:17) with Fraction A and Fraction C in the same ISCOM particle thus corresponding roughly to the 703 mixture in the Cox patent.

20. As shown ISCOM formulations containing saponin Fraction A and Fraction C

formulated in the same particles exert toxicity in the same range as ISCOMs containing 100% of the toxic component Fraction C alone. On the other hand, the same amount of saponins mixed together but formulated in separate particles does not exert side-effects over the formulation containing 100% of the non-toxic component Fraction A. Lethargy score at 100 µg for ISCOM -C is 5, for ISCOM-AC (mixed as in the Cox patent) is 6 and according to the present invention ISCOM- A+ ISCOM- C is only 2.5.

21. Enclosure 2 shows cytotoxity and lethargy tests with ISCOM matrix. From table 1 and figure 2 of this test, it is evident that Matrix 703 (Fraction A and Fraction C in the same particle) has a LC50 value of around 18.7 in the cytotoxity test. Matrix MIX (Fraction A and Fraction C in different ISCOM matrix particles) has a LC50 value >240 in the cytotoxity test.

22. Figure 1 of Enclosure 2 shows that lethargy is lower for matrix mix according to the present invention (M-A(10) + M-A (1) and (2) respectively than for matrix containing Fraction C only.

23. In an extended toxicity study (Enclosure 3) it was shown that mice (20 g) tolerate doses in the level of 100 µg or higher without dying or showing significant discomfort provided that the saponins were separately formulated in ISCOM-Matrix. This gives a value of 5 mg/kg of Fraction A and Fraction C in separate

ISCOM particles according to the present invention being well tolerated compared with 1,4 mg/kg Fraction A and Fraction C in the same ISCOM particle being lethal according to the Cox patent.

24. The lethal dose for Balb/c mice were substantially (five-fold or more) reduced when comparing administration of conventional mixtures of Fraction A and Fraction C together in matrix according to the Cox patent compared with comparable amounts of mixtures of Fraction-A matrix and Fraction C matrix according to the present invention.

Antibody Response

25. In Example 4 page 19 mice were immunized with products 1-7 of Table 2 and IgG1 and IgG2a were analyzed. A mixture of particles (MIX) according to the present invention enhances the same level of IgG1 antibody (Fig 2A) to PR8 micelles as the same dose of Fraction A and Fraction C in the same proportions when incorporated in the same particle (for example, CONV particles as in the Cox patent). However, higher levels of IgG2a antibody antibodies were enhanced by the MIX formulation (Fig 2B). The groups 1- 3 (MIX according to the invention) were compared with the group 7 (CONV; the 703 product according to the Cox patent). Mice in group 7 injected with a low dose of 10µg (CONV 70:30), i.e. the dose the mice can accept, responded with a potent IgG1 (Fig 2A) response, but a

low IgG2a response (Fig 2B).

26. Thus, the invention with a matrix formulation having a mixture of matrix particles comprising different saponin fractions according the present invention can be given in high doses without side effects while enhancing the antibody response to higher levels than those with than the CONV matrix. The IgG2a response is particularly enhanced. The IgG2a response is a TH1 response and is particularly important for defense against intracellular parasites such as viruses.

Cell mediated immune response

27. Fig. 3 shows the cell mediated immune response measured as the production of the cytokines IL-5 (Figure 3A) and IFN-γ (Figure 3B) by spleen cells collected week 6 after immunization as described in the legend to Fig 2, after stimulation in vitro with influenza virus micelles as described in the text.

28. The CONV matrix formulations as in the Cox patent have inferior capacity to enhance cell mediated immunity in the doses tolerated than the MIX formulations according to the present invention (Figs 3 A and B). The MIX formulation (92:8, group 2) enhances considerably higher IL-5 levels than the CONV (70:30.group 7 COX 703) or the 100% QHC matrix. formulation (group 6). The mix (92:8, group 2) formulation also enhances the IFN-γ considerably better than the QHC 100%

matrix (group 6) or the CONV (70:30, group 7) formulation. Production of IL-5 and 5 and IFN-γ is an indication of a concomitant balanced TH1 and balanced TH1 and Th1 type H1 type of response, i.e., an overall adjuvant effect.

Synergistic effect

29. Fig 5 shows synergistic effects of Fraction A and Fraction C matrices when supplemented to OVA to enhance the antibody response in Balb/C mice (see text). The dose of Fraction A and C matrices ranged as follows in group 1, no A or C; Gr. 2, 0.3µg A no C; Gr. 3, 0.3µg A + 2µg C; Gr. 4, 10µg A no C; Gr. 5, 10µg A 2µg C. The dose of OVA was 10µg. There were 8 mice per group, which were immunized twice 4 weeks apart subcutaneously with respective formulation. The antibody were measured by ELISA against:

30. Total IgG 3 weeks after the first immunization; Figure 5A
IgG2a 2 weeks after the second immunization; Figure 5B
IgG1 2 weeks after the second immunization; Figure 5C

31. After the first immunization, no antibody response was recorded in mice receiving non-adjuvanted OVA or OVA adjuvanted with 0.3µg of Fraction A matrix with and without 2µg of Fraction C matrix. (Fig 5A).

32. After the second immunisation a low response was detected in 3 out of 8 mice immunized with non-adjuvanted OVA in the IgG1 subclass but no response was recorded in the IgG2a subclass. Neither was antibody responses recorded with the lowest adjuvant doses of Fraction A matrix Fraction A matrix i.e. 0.3µg with and without 2µg of Fraction C matrix. There was a clear enhancement of the antibody response in the IgG2a subclass when the low dose of 2µg Fraction C was added to the 10 µg of Fraction A (Fig 5B).

Summary

33. The toxicity is considerably reduced with the compositions according to the present invention as compared to invention of the Cox patent. Thus, as described above the invention of the present application (saponin fractions in different ISCOM particles) obtains a lethal dose in mice that is at least five fold higher than when the saponin Fractions are integrated into the same particles as in the Cox patent. To date doses up to 100µg of saponins in the composition of the present invention have been repeatedly used without discomfort and or single death in mice.

34. Furthermore, there is a synergistic effect in the adjuvanted antibody response. First, the magnitude of the IgG1 response is increased. Second there are pronounced features of both TH1 (IFN gamma and IgG2a antibody subclass) and

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35. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dated: May 28, 2008
Uppsala, Sweden

Karin Lovgren Bengtsson, Ph.D.